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THE UNIVERSITY OF ALBERTA

THE EFFECT OF DIETARY PROTEIN CONCENTRATION
ON ZINC METABOLISM
IN RUMINANT HOLSTEIN CALVES

by

JO MARY WELCH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1988

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(SIGNED) ...*J. Welch*...

PERMANENT ADDRESS:

...*R.R. #4*...

...*Sherwood Park*...

...*Alberta*...

Date: ...*Oct. 14, 1988*...

THE UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE EFFECT OF DIETARY PROTEIN CONCENTRATION ON ZINC METABOLISM IN RUMINANT HOLSTEIN CALVES submitted by JO MARY WELCH in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

.....*James*.....
Supervisor

.....*Elizabeth A. Donald*.....

.....*J.R. Thompson*.....

.....*G.A. Matheson*.....

.....

Date: *October 14* 1988

ABSTRACT

Zinc is an essential nutrient for all animals.

Although dietary protein concentration influences zinc metabolism in monogastrics, its effect on the metabolism of zinc in ruminants has received scant attention. Thus, this experiment was designed to examine the effect of dietary protein concentration on zinc absorption and excretion in calves.

Absorption and excretion of stable zinc and ^{65}Zn were studied in three ruminant Holstein calves in a 3 X 3 Latin Square design. The calves were fed diets containing 35 $\mu\text{g Zn/g}$ dry matter and 8%, 15% and 20% crude protein. The major ingredients of the basal diet were barley green feed and dried beet pulp. Protein concentrations were adjusted with dried egg white and treated soy flour.

^{65}Zn was injected intravenously after a 10 day dietary adjustment period and the changes in specific radioactivity of plasma, feces and urine were monitored for 5 days. Endogenous zinc loss and true absorption of zinc were estimated, using compartmental analysis. Different possible models were considered, and a three-compartmental model was chosen as the most suitable representation of the data.

Plasma zinc concentration and apparent and true absorption of zinc were unaffected by dietary protein concentration. Endogenous zinc excretion was also not

affected by changes in dietary protein, whether expressed in absolute terms, or as a fraction of total zinc excretion. It therefore appears that dietary protein concentration may exert no effect on the zinc metabolism of Holstein calves.

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CHAPTER I.

INTRODUCTION

ZINC IN BIOLOGY

A Historical Review

The importance of zinc in biological systems has long been recognized. Although research into the role of zinc in ruminant metabolism began only in 1960, earlier and concurrent studies of zinc in non-ruminants have greatly enhanced the progress of zinc research in ruminants. Zinc was first shown to be necessary for the growth of living matter in 1869 when Raulin demonstrated that Aspergillus niger required zinc for growth. In 1877 Lechartier and Bellamy reported the presence of zinc in animal carcasses. Also in 1877, Raoult and Breton discovered zinc in human liver.

Five decades later, Lutz (1926) found zinc to be ubiquitous in all organs of rats, cats and man. Industrial growth in the 1920's resulted in a mysterious illness termed "metal fume fever". Investigations into the cause of this disease lead Collier and Drinker (1926) to develop a clinical method for zinc analysis in organs. Upon noticing that zinc was prevalent in biological tissue, they hypothesized that zinc must be involved in important biological functions. However, a mechanism by which zinc may function in biology was not elucidated until Keilin and

Mann in 1940 reported their discovery of the first known zinc enzyme, carbonic anhydrase.

In 1934 Todd et al. first demonstrated that zinc was essential for growth and hair maintainance in rats. Shortly thereafter, Sutton and Nelson (1937) reported that high dietary zinc levels can have deleterious effects on growth and reproduction in rats. Also in 1937, Hove and coworkers described dermal lesions in zinc deficient rats. However, prior to 1955, zinc deficiency was considered a disease that existed solely in laboratory animals on extreme diets and not under normal conditions (Dukes 1955, Maynard and Loosli 1956, Underwood 1956). New advances in zinc research changed that attitude. In 1955 Tucker and Salmon discovered that parakeratosis, a disease then common in swine reared on diets containing high levels of plant protein supplements, was curable by adding zinc to the diets. Further research showed that zinc deficiencies also occurred in poultry (O'Dell et al. 1958), humans (Prasad et al. 1961) and dogs (Robertson and Burns 1963).

Zinc deficiency in ruminants was first observed in 1960. Legg and Sears (1960) reported that parakeratosis in Guyanian cattle could be prevented and cured by zinc supplementation, while Miller and Miller (1960) experimentally developed and characterized zinc deficiency in calves. In 1961 Haaranen and Hyppola were able to reverse such symptoms of zinc deficiency as skin lesions,

poor reproductive performance and low milk yield by feeding supplementary zinc to Finnish dairy cattle. Soon after, Grashuis (1963) reported zinc deficiency in Dutch cattle, although the accuracy of that report has been contested (Hartmans 1965).

Zinc research before 1960 was severely limited by imprecise analytical techniques. Vast improvements in the spectrophotometric analysis of zinc, first described by Allen (1961), revolutionized subsequent research. Such technical improvements as well as increased interest in the medical implications, functions, metabolism and effects of varying dietary levels of zinc have resulted in a much better understanding and awareness of zinc metabolism and nutrition in the last quarter century.

The Importance of Zinc

Zinc is an essential component of over 200 enzymes and, as such, may be necessary for the correct functioning of all metabolic processes in the body (Vallee 1983). Zinc has been shown to be indispensable for both cellular growth and differentiation (Vallee and Falchuk 1981) and total body growth (Underwood and Somers 1969). Retardation of growth in zinc deficient animals can often be attributed to anorexia (Wallwork et al. 1981) which in turn may be due to alterations in the parotid salivary glands and their proteins (Johnson and Alvares 1984). Sufficient quantities

of dietary zinc are also necessary for dermal integrity (Ott et al. 1965a), wound healing (Miller et al. 1965) and cartilage and bone formation (Starcher et al. 1980).

Zinc plays an integral role in the healthy functioning of the mammalian immune system (Beisel 1982). This may be due to the requirement of DNA synthesis for adequate zinc (Fujioka and Lieberman 1964). DNA synthesis, in turn, is a prerequisite for synthesis of T cells in the thymus (Fraker et al. 1977), proliferation of B cells in the spleen and lymphocyte synthesis (Frost et al. 1977). Zinc is also necessary for the desaturation of linoleic acid (18:2w6) to linolenic acid (18:3w6), which is the precursor of prostaglandins (Hwang et al. 1982). The synthesis of prostaglandins, which are important factors in the immune response (Song and Adham 1985), is altered in zinc deficient rats (Chanmugam et al. 1984).

Zinc is affected by, and exerts an effect on, the endocrine system. It elicits a hyperglycemic effect on the body, possibly by functioning as an alpha and beta blocker, by increasing glucagon release (Etzel and Cousins 1983), and by regulating the synthesis, storage, secretion (Figlewicz et al. 1984) and activity (Jendryczko and Kucharz 1984) of insulin. Evidence suggests that both glucocorticoids (Etzel and Cousins 1981) and the catecholamines, epinephrine and norepinephrine (Brady and Helvig 1984), induce elevated hepatic zinc levels.

Zinc status may also affect secretion of aldosterone by regulating the production of angiotensin converting enzyme (ACE) (Reeves and O'Dell 1985). ACE facilitates the transformation of angiotensin I into angiotensin II which in turn stimulates the production of aldosterone. Perhaps it is through this control of aldosterone levels that zinc is involved in the movement of water across cell membranes (Bettger et al. 1979).

Researchers have suggested that some of the functions of somatotropin (Cheruvanky et al. 1982), gonadotropin (Scott et al. 1984), insulin-like growth factor (somatomedin C) and testosterone (Ghavami-Maibodi et al. 1983) are mediated through their effects on zinc metabolism. Because adrenocorticotrophic hormone (ACTH) levels are also reported to be zinc dependent (Sandstead et al. 1967), it is likely that there is adreno-cortical control of zinc in the body.

Adequate zinc is also necessary for reproductive performance. Zinc deficiency can result in testicular atrophy (Miller and Miller 1962) and decreased spermatogenesis (Underwood and Somers 1969). Females also require adequate dietary zinc to maintain normal estrous cycles (Hurley 1969).

Maternal zinc deficiency can result in fetal resorption (Hurley 1969), poor fetal growth (Fosmire et al. 1977), and dystocia (Dura Trave et al. 1984). Zinc deficiency during

prenatal life can also result in such central nervous system damage as reduced DNA synthesis in the fetal brain (Eckhert and Hurley. 1976) and alterations in neurochemistry (Geiger et al. 1982). It is possible that these changes result in the facial deformities, hydrocephalus, and anacephaly that have been reported with severe, in utero zinc deficiencies (Hurley and Shrader 1972). The teratogenic effects of maternal zinc deficiency often resemble those seen in conjunction with copper toxicity. Indeed, these effects appear to be more likely due to copper-zinc antagonism (Reinstein et al. 1984) than to abnormalities in DNA synthesis (Hurley 1969). Adequate zinc in utero is also necessary for the proper functioning of the eye (Leure-duPree and McClain 1982), as the eye and optic nerve are the neural tissues most sensitive to preparturitory zinc deficiency (Kasarskis 1984).

ZINC METABOLISM

Site of Absorption

The principle site of zinc absorption is likely the ileum in both rats (Kowarski et al. 1974, Antonson et al. 1979) and cattle (Miller and Cragle 1965). The alkaline condition of the ileum results in the formation of zinc complexes (Bremner 1970) that may act as carriers for zinc through the mucosal membranes (Williams 1981). A substantial amount of zinc is also absorbed in the

duodenum, jejunum (Antonson et al. 1979, Davies 1980) and possibly in the abomasum (Miller and Cragle 1965). Little or no zinc absorption occurs in the caecum or colon in rats (Methfessel and Spencer 1973) or calves (Pate et al. 1970). Also, zinc is not absorbed from the stomach of sheep (Ivan 1987).

Mechanism of Absorption

The mechanism by which zinc is absorbed into intestinal cells is not yet clear. It has been suggested that zinc is absorbed in three forms: (1) as free zinc, (2) as a zinc metalloprotein and, (3) as a low molecular weight zinc chelate (Cousins 1979). More recent research has shown both carrier mediated and nonmediated mechanisms to be involved (Menard and Cousins 1983a). In vitro and in vivo studies indicate that the carrier mediated component is saturable and may be the exclusive mechanism of zinc absorption in zinc deficient enterocytes. The nonmediated component, which can occur in zinc sufficient intestinal cells, is considered a nonsaturable mechanism (Menard and Cousins 1983a, Steel and Cousins 1985).

The effect of chelation on zinc absorption is less certain. Savage (1972) reported that chelation improved the availability of dietary zinc to poultry and rodents. Ho and Hidioglou (1977), however, found that both chelation and sequestration depressed plasma zinc

concentrations in sheep.

The identity of a carrier or carriers of zinc across the mucosal membrane has been investigated. Early researchers observed that a gastric influx of zinc resulted in elevated levels of a low molecular weight ligand in both the intestinal lumen (Starcher 1969) and the cytosol of enterocytes (Van Campen and Kowalski 1971). Others reported that zinc in the mucosal cytosol is bound to proteins of both low and high molecular weight (Hoardley and Cousins 1985). The low molecular weight zinc binding ligand was identified as metallothionein (Evans et al. 1970) and hypothesized to be the absorptive carrier of zinc (Kowarski et al. 1974). However, metallothionein is not solely a zinc ligand; it also binds readily with both copper and cadmium (Starcher 1969, L'Abbe and Fischer 1984). Although copper can compete with zinc for absorption, the large amount of zinc normally absorbed in the presence of copper (Cotzias et al. 1962) rules out a non-specific protein such as metallothionein as being a zinc-specific carrier. Elevated zinc concentrations in the intestinal lumen induce increases in concentrations of metallothionein. The metallothionein then appears to bind zinc within the enterocyte and to reduce the rate of passage of zinc through the basolateral membrane. Metallothionein thus appears to act more as a zinc storage protein than as a regulating agent (Olafson 1983).

Other low molecular weight zinc binding ligands that have been suggested include N,N,N'-trimethyl-1,2-ethanediamine (Hahn et al. 1976), prostaglandin E₂ (Evans and Johnson 1977, Song and Adham 1978), citric acid (Hurley et al. 1979), picolinic acid (Evans et al. 1975) and proteolytic products of metallothionein (Cousins et al. 1978). Although many of these suggestions may have some merit, most have also been disputed. N,N,N'-trimethyl-1,2-ethanediamine and breakdown products of metallothionein have since been considered to be artifacts of the purification process (Cousins 1979, Evans 1980). An oxygen-rich molecule such as prostaglandin E₂ is a chemically unsuitable candidate to be a zinc binding ligand (Sigel and McCormick 1970). Moreover, Smith et al. (1978) found that prostaglandin E₂ does not influence zinc absorption in vitro. Menard and Cousins (1983b) did not find citrate to enhance zinc absorption. Picolinic acid was reported by Antonson et al. (1979), Menard and Cousins (1983b) and Hill et al. (1988) to depress zinc absorption in rat, pig and poultry intestines respectively. However, the report by Smith and colleagues (1979) that the low molecular weight zinc binding ligand does not affect zinc absorption could dampen the search for the ligand's identity.

Other compounds may play a role in zinc absorption. Although zinc flux is likely the result of an active

transport process requiring adenosine triphosphate (ATP) (Kowarski et al. 1974), Menard and Cousins (1983a) did not find that ATP stimulated zinc uptake into isolated brush border membranes. Kowarski et al. (1974) reported that increased zinc absorption in vitro was facilitated by ambient sodium, oxygen, a metabolizable hexose and vitamin D. Steinhardt and Adibi (1984), however, found that the presence of sodium resulted in decreased zinc absorption in vivo. They also reported that the absorption of zinc was selectively stimulated by such organic solutes as glycylleucine and glucose.

Certain amino acids may also increase zinc absorption. Tryptophan, histidine, proline and cysteine may enhance zinc absorption (Wapnir and Stiel 1985) while glycine, leucine (Steinhardt and Adibi 1984) and methionine (Hill et al. 1988) are reported to have no effect. Although some of these organic compounds may indeed enhance and facilitate zinc absorption, the mechanism by which they act still remains unclear.

Transport and Storage

In vitro studies on zinc adequate animals suggest that zinc transport through the basolateral membrane of serosal cells may be a rate limiting step (Davies 1980). However, some in vivo research on zinc deficient animals has shown that absorbed zinc is rapidly transferred to the

capillaries (Pate et al. 1970, Antonson et al. 1979, Davies 1980). Although newly absorbed and bound zinc in the enterocyte is not re-excreted to the lumen (Davies 1980), the passage of dead, zinc-containing mucosal cells into the gut lumen may be a major source of fecal zinc (Miller 1969).

Zinc departing from the intestinal serosa travels via capillaries to the superior mesenteric vein which then joins the splenic vein to form the portal vein. The portal vein then delivers the zinc to the liver where it is either stored or travels through the hepatic vein to the inferior vena cava. Blood in the inferior vena cava arrives at the heart and is then circulated to all areas of the body (Gray 1977).

Albumin, the major carrier of bound zinc in blood serum (Giroux and Henkin 1972), is generally believed to be the plasma protein that transports zinc from the intestines via the portal system (Evans et al. 1975, Cousins 1979). However, Evans and Winter (1975) reported that the major portion of nascent zinc in rat portal plasma was bound to transferrin. The finding by Levine et al. (1972) that apotransferrin binds to intestinal serosal cells lent further strength to the transferrin concept. Evans (1976) subsequently hypothesized that portal apotransferrin interacts with the basolateral membrane to remove zinc from binding sites: the transferrin-zinc complex then leaves the

membrane and is transported through the portal vein to the liver, where the metal is incorporated into albumin and other zinc binding proteins. That hypothesis, however, was tested and refuted by Smith et al. (1979), who found that 94% of absorbed zinc is transported in portal blood by albumin.

Approximately two thirds of the zinc in blood serum is loosely bound to albumin (Giroux and Henkin 1972). Because albumin may be the only serum macromolecule that binds exchangeable zinc (Giroux and Henkin 1972), this fraction is likely the most metabolically important.

Alpha₂-macroglobulins firmly bind 30 to 40% of the total zinc in serum (Parisi and Vallee 1970), while cysteine and histidine loosely bind a further 2% of the zinc (Giroux and Henkin 1972).

Zinc concentrations vary among tissues. The highest zinc levels in normal calves fed normal diets adequate in zinc are generally in the liver (Miller 1969). The zinc content of calf muscle, pancreas and digestive tract tissue is also high. Cartilage, lung and testicular tissue contain intermediate concentrations of zinc, while the spleen, kidneys and bones contain somewhat lower zinc concentrations (Miller 1969).

Differences between tissues and between species are apparent in the turnover rates of zinc. The plasma, liver and spleen of calves appeared to absorb and exchange zinc

more rapidly than did other body tissues (Miller et al. 1967), while the kidney, heart, lung, testicles and skin of both goats and calves also exhibited both a high affinity for and turnover of zinc (Miller 1969). Neathery and coworkers (1973a) reported that zinc concentrations in the rib cartilage and rumen walls of dairy cows responded the most quickly to changes in dietary zinc intake. In rats, turnover of zinc is rapid in bone and slow in the spleen (Giugliano and Millward 1984). Both Giugliano and Millward (1984) and Peirce et al. (1985) concluded that zinc deficient rats selectively move zinc from bone to muscle, possibly to allow growth. Others reported that mobilization of skeletal zinc in rats is enhanced by a high calcium status and conditions that promote bone resorption (Tao and Hurley 1975).

The age of an animal can also affect its zinc metabolism, as the total dietary zinc absorbed by rats (Gunn et al. 1963) and cattle (Miller and Cragle 1965) is reported to decrease with age. This age factor can, however, disappear in zinc deficient animals (Miller 1969).

Zinc Excretion

Most secreted endogenous zinc is delivered to the small intestines via the capillaries of the superior mesenteric artery. Smaller quantities of zinc arrive in the duodenum

through the pancreatic and common bile ducts (Berger and Schneeman 1986) and by secretion into the stomach (Ivan 1987). The site of secretion of most endogenous zinc is generally believed to be the proximal intestine (Miller and Cragle 1965, Ivan 1987), although one in vitro study reported that serosa to mucosa transfer of zinc was most rapid in the jejunum and ileum of rats (Kowarski et al. 1974). Endogenous zinc secreted into the proximal intestine can be resorbed in the distal intestine (Miller 1969).

Zinc excretion is negligible in urine of mice (Cotzias et al. 1962), rats (Bloxan et al. 1984), sheep (Suttle et al. 1982), and cattle (Miller and Cragle 1965) for several possible reasons. Bloxan et al. (1984) reported that only 0.2% of plasma zinc is non-protein bound and suggested that only this fraction can be eliminated in urine. In addition, Giroux and Henkin (1972) offered the possibility that 75% of zinc entering the kidney is resorbed. They suggested that, since zinc-amino acid complexes easily cross the renal glomerulus, about 1.1 mg zinc per 100 liters of filtrate should enter the urine. However, only 0.27 mg zinc per 100 liters appeared.

Zinc Homeostasis

The relationship between the level of zinc in the diet and zinc metabolism has been extensively examined. Cotzias

and coworkers (1962) were the first to provide convincing evidence that zinc is under homeostatic control. Whether zinc homeostasis occurs at sites of absorption or excretion, however, remains controversial.

The ability of the body to regulate zinc absorption homeostatically has been reported in humans (Istfan et al. 1983), rats (Hallmans et al. 1987), mice (Cotzias et al. 1962), horses (Schriver et al. 1980), sheep (Suttle et al. 1982) and cattle (Miller et al. 1968). Metallothionein has been implicated as the controlling factor in zinc transfer from the intestinal lining to the rest of the body. Zinc absorbed into the enterocyte induces metallothionein formation. The metallothionein then binds the zinc, thereby allowing less zinc to pass from the intestines into the blood (Menard et al. 1981). Because the greater the body content of zinc the more metallothionein is induced, enterocytes of zinc deficient animals contain a very low concentration of metallothionein (Olafson 1983).

Intestinal zinc excretion may also be under homeostatic control. Most tissues in zinc deficient animals selectively retain zinc (Miller et al. 1967); control of zinc excretion may also occur at the site of intestinal secretion (Cotzias et al. 1962). In addition, lactating animals placed on a zinc inadequate diet exert strong control on body zinc by decreasing zinc secretion into milk and by excreting less zinc in the feces than do non-lactating animals (Neathery et al. 1973b).

ZINC NUTRITION

Requirements

Zinc requirements for dairy cattle are contained in the National Research Council's publication: Nutrient Requirements of Dairy Cattle (1978). Although most of the nutrient recommendations are specific for age, sex, and reproductive and lactational stages, a minimum dietary zinc content of 40 $\mu\text{g Zn/g DM}$ is recommended for all dairy cattle diets. A maximum zinc content of 500 $\mu\text{g Zn/g DM}$ is recommended although it is acknowledged that mature cattle can tolerate 1000 $\mu\text{g Zn/g DM}$.

The minimum zinc requirement was derived primarily from research by Miller and coworkers (1965b), Stake et al. (1973) and Voelker et al. (1969). However, research which suggests lower requirements for zinc in dairy or beef cattle is also cited (Miller et al. 1963, Mills et al. 1967a, Perry et al. 1968, Zurcher 1970, Neathery et al. 1973a). Thus it is apparent that the dietary zinc concentration recommended by the NRC (1978) may be above the minimum zinc requirement for most dairy cattle.

Absolute zinc requirements are not known; establishing them is a difficult task. Presumably, an animal's requirement for a nutrient is marginally greater than the level that would produce signs of ill health. Although the

symptoms of a classic zinc deficiency are well documented (Miller and Miller 1960, Mills et al. 1967a) there is little agreement on the identity of, or method of quantifying the earliest or most marginal signs. Blackmon et al. (1967) suggested that dermal lesions are best attributed to zinc deficiency if they respond to zinc supplementation. This method merits practical consideration but lacks accuracy. Also, failure to respond to zinc supplementation could occur in a multi-deficiency situation because another deficiency could be the limiting factor (Field, 1981). Mills et al. (1967a) concluded that plasma zinc concentrations repeatedly below $0.4 \mu\text{g/mL}$ indicate a zinc deficient state in calves. However, plasma zinc levels are not always indicative of zinc status (Beeson et al. 1977, Miller 1970). Numerous other factors influence the level of zinc in blood: any stress to an animal can cause a depression, whereas sample hemolysis increases plasma zinc concentration (Underwood, 1979). Zinc deficiency can also result in depressions in total serum proteins, carbonic anhydrase activity (Ott et al. 1965a) and alkaline phosphatase (Kirchgessner and Roth 1981) although these parameters are subject to a wide range of individual variation. Although a depression in growth rate has been postulated as an indicator of zinc deficiency (Perry et al. 1968), it is a method that is applicable only to immature animals and often occurs only after other

symptoms of zinc deficiency have become apparent (Mills et al. 1967a).

Zinc availability can vary with animal and dietary factors. Animal factors may include genetics (Kroneman et al. 1975), sex (Ott et al. 1966), age (Stake et al. 1973), reproductive status (Dufty et al. 1977), and individual variation (Ott et al. 1965b, Mills et al. 1967a). Zinc absorption may be higher in dairy cattle fed semi-purified diets (Mills et al. 1967a, Neathery et al. 1973a), but this factor has not been studied in isolation from other dietary differences. The form of supplemental zinc ingested does not seem to affect zinc bioavailability in either humans (MacFarlane et al. 1976) or dairy cattle (Kincaid 1979).

Other dietary constituents interact with zinc. The effects of protein, energy, minerals and vitamins on zinc absorption and metabolism have been investigated; each of those factors is discussed below.

Effects of Protein Level and Source

Many researchers have investigated the effect of quantity and source of dietary protein on zinc absorption and metabolism in monogastrics. Comparisons of protein-zinc interactions between non-phytate containing protein sources are sparse. Diets that contain intrinsic (such as soybean products), or extrinsic phytic acid strongly inhibit zinc absorption in rats (Hardie-Muncy and

Rasmussen 1979, Forbes et al. 1979, Meyer et al. 1983), swine (Oberleas et al. 1960, Smith et al. 1962), quail (Fox et al. 1985), humans (Solomons et al. 1982) and preruminant lambs (Hill and Walker 1983). However, phytic acid does not impair zinc absorption in ruminants (Ott et al. 1964), because rumen microorganisms readily degrade phytate (Raun et al. 1956).

A diet containing high levels of animal proteins can increase apparent absorption of zinc in rats (Snedeker and Greger 1983), chickens (Lease et al. 1960) and swine (Pond et al. 1985). True absorption of zinc in rats (Greger and Mulvaney 1985) is also increased with greater dietary protein concentrations. Similarly, a protein deficient diet can result in lower retention of zinc in rats, due to both a decrease in zinc absorption and an increase in the excretion of endogenous zinc (Van Campen and House 1974, Hooda et al. 1984a).

High protein diets have been reported to cause increased excretion of urinary zinc in rats (Hooda et al. 1984b) and humans (Greger and Snedeker 1980, Colin et al. 1983, Mahalko et al. 1983). That increase is likely of little significance to total zinc balance, however, as urine is a very minor route of endogenous zinc excretion (Miller et al. 1966, Suttle et al. 1982).

An increase in the dietary content of digestible protein can increase the amount of carboxypeptidase, a

proteolytic enzyme secreted by the pancreas (Mills et al. 1967b, Berger and Schneeman 1985). It also results in an increase in zinc and protein secretion. Although the significance of the zinc - carboxypeptidase association is as yet unclear, Antonson et al. (1979) found that pancreatic secretions did not affect zinc absorption.

The effect of dietary protein levels on zinc absorption and metabolism in ruminants has received scant attention. Miller and coworkers (1969) investigated the effects of a high protein level on calves and found it to have no effect on hair and serum zinc. In a concurrent study, Martin et al. (1969) reported that a low protein diet reduced zinc levels in serum but increased zinc concentration in hair.

Effects of Dietary Energy

The negative effects of a low dietary energy level on growth rate, protein deposition and nitrogen retention is well established (Munro 1964). Because these effects are similar to symptoms of zinc deficiency, the energy-zinc relationship has been examined. Steinhardt and Adibi (1984) reported enhanced zinc absorption in the presence of glucose in humans. However, this phenomena is unlikely to occur in ruminants, because glucose is not an end product of carbohydrate digestion (Halliwell 1961). Despite a report by Martin et al. (1969) that a low energy diet can cause a slight depression in the zinc concentration of

blood serum in calves, other reports suggested that energy intake does not influence zinc absorption in bovines (Miller et al. 1969, Stake et al. 1973).

Effects of Copper

Early insight into interactions between zinc and other minerals was provided in 1950 by Gray and Ellis who stated that "the demonstration of such relationships as . . . between copper and zinc . . . emphasize the necessity for a consideration of the levels of all minerals in the diet before determining the requirements of any one". Later research validated their concerns. Antagonism between dietary concentrations of zinc and copper has since been reported in mice (Cotzias et al. 1962), rats (Van Campen and Scaife 1967), quail (Hamilton et al. 1979), humans (Fischer et al. 1984), sheep (Weber and Leaver 1980) and cattle (Koetsveld and Lehr 1961).

The mechanism of the zinc-copper interaction is uncertain. A competitive binding in the intestinal epithelium was hypothesized by Van Campen (1968) and Weber and Leaver (1980). Cymbaluk et al. (1981) suggested that the same homeostatic mechanism controls both zinc and copper status. Zinc, but not copper, apparently induces metallothionein production in the cytosol (Fischer et al. 1983). The metallothionein then sequesters both copper and zinc, rendering them less available for serosal transfer

(Fischer et al. 1983). Further evidence for this comes from Oestreicher and Cousins (1985) who reported that zinc supplemented rats transfer less copper to the portal system. However, the copper status of an animal appears to have an effect on zinc-copper interactions. Fischer et al. (1983) reported that most of the intestinal copper in enterocytes of animals with a low copper status was complexed with metallothionein. As dietary copper was increased, proportionally more copper was found to be affiliated with a high molecular weight protein. Furthermore, the copper bound to the high molecular weight protein was not affected by zinc status and was readily transferred to the portal system.

The effect of copper on zinc metabolism therefore appears to be dependent on the levels of zinc and copper in the diet. Zinc deficiency can be induced in animals fed a diet high in copper and low, but not necessarily deficient, in zinc (Suttle and Mills 1966). Animals receiving the NRC recommended concentrations of both zinc and copper, however, are unlikely to suffer from the adverse effects of a copper-zinc imbalance.

Effects of Other Minerals and Vitamins

High dietary calcium concentrations have been demonstrated to adversely affect zinc status in rats (Morris and Ellis 1980), dogs (Robertson and Burns 1963),

pigs (Hsu et al. 1975), sheep (Suttle and Field 1970), beef cattle (Perry et al. 1968) and dairy cattle (Haaranen 1965). However, Spencer et al. (1965, 1984) and Pond (1983) could not demonstrate calcium-zinc antagonism in humans or lambs, respectively. Animals affected by calcium-zinc antagonism can exhibit decreased zinc absorption (Adham and Song 1980), decreased bone zinc (Hsu et al. 1975, Forbes et al. 1984), decreased hair zinc (Perry et al. 1968) and increased zinc excretion (Suttle and Field 1970). It has been suggested that the same factors responsible for zinc homeostasis are also involved in controlling calcium status (Song et al. 1985). Thus, animals on a marginal zinc diet can exhibit symptoms of zinc deficiency if the ratio of calcium to zinc is too high (Robertson and Burns 1963). To avoid this problem in dairy cattle, Haaranen (1963) proposed that cattle on a 45 μg Zn/g DM diet should receive an additional 16 μg Zn/g DM for every 0.1% increase in calcium above 0.3% in the diet (DM basis).

Interactions between zinc and several other minerals have been investigated. High dietary levels of magnesium depressed tibia zinc (Forbes et al. 1984). Both ferric and ferrous iron supplements depressed plasma zinc concentrations (Solomons et al. 1983a) while ferrous iron decreased zinc absorption (Solomons et al. 1983a). Solomons et al. (1983b) reported that dietary tin did not

affect zinc absorption while Johnson and Greger (1984) found that high doses of tin resulted in greater fecal excretion of zinc. Interactions between zinc and nickel were reported to be tissue specific (Reichlmayr-Lais et al. 1985); gallium did not affect zinc metabolism (Cotzias et al. 1962).

Lead and cadmium are considered to be unnecessary and deleterious elements in animals. Lead and zinc have been reported to have both an additive effect (Hsu et al. 1975) and a slightly antagonistic effect (White et al. 1985) on each other. Cadmium, however, is a zinc antagonist: supplemental cadmium can accelerate zinc excretion (Cotzias et al. 1962) and supplemental zinc can reduce the cadmium burden in body tissues (Lamphere et al. 1984).

Certain vitamins may affect zinc metabolism. Prasad and coworkers (1982) investigated the effects of vitamins B6 (pyroxidine) and B1 (thiamine) on zinc metabolism. They concluded that vitamin B6 deficiency increased intestinal absorption of zinc while a vitamin B1 deficiency decreased it. Interactions between zinc and vitamin A have also been researched. Although Sarawat and Arora (1972) and Arora et al. (1973) reported that zinc supplements ameliorated vitamin A deficiency symptoms in lambs, Smith et al. (1977) found no definitive correlation between vitamin A and zinc status in rats.

Implications in Western Canada

Most feedstuffs in Alberta and Saskatchewan contain insufficient zinc concentrations to meet the dietary zinc requirements of cattle (Christensen et al. 1978, Westra, 1982). It is therefore surprising that cases of clinical zinc deficiency are not commonly reported. The reason may be that marginal zinc deficiency is often difficult to detect. However, it is entirely possible that the high protein content of most feedstuffs grown in the prairies of western Canadian lowers the zinc requirement of cattle. The present study was designed to investigate the effect of dietary protein level on zinc metabolism in weaned, Holstein calves.

METHODOLOGY IN ZINC NUTRITION AND RESEARCH

Balance Studies

Balance studies provide a simple approach to the determination of relative availability and utilization of nutrients by animals. Information from balance studies is also used to establish the nutrient requirements of animals. Apparent absorption is one nutritional parameter that is readily calculated from balance trial data. It is calculated by subtracting the fecal excretion of a nutrient from its intake, and is expressed as a percentage of intake of that nutrient. A low apparent absorption may indicate that the form of the nutrient ingested is not readily

available to the animal. Results of mineral balance trials, however, can indicate more about the nutritional status of the animal than about its requirements. A major concern with the use of balance trials as a tool to assess the requirement for a mineral is that steady state conditions are difficult to achieve experimentally. For example, regulation of the balance of trace minerals such as zinc is based on the need of an animal for that nutrient (Hallmans et al. 1987). An animal with a low zinc status will absorb and retain more zinc, and therefore indicate a higher requirement for zinc, than will an animal with a higher zinc status (Mertz, 1987). Results from balance trials can therefore be misleading if a nutritional steady state is not achieved before and maintained throughout the experiment.

A high degree of technical accuracy is also difficult to achieve in a balance trial. The amount of feed ingested is often overestimated because of feed wastage. The feed wasted is most commonly the finer portions that may be higher in the nutrient under examination. Total excretion is generally difficult to obtain and is therefore often underestimated (Avioli 1980). Technical difficulties inherent in balance studies often result in higher estimates of nutrient absorption than are achieved by carcass retention and isotope trials (Duncan 1967, Davidson and Williams 1968). In addition, balance trials cannot, by

themselves, be used to calculate estimates of endogenous excretion (Asplund 1979).

Isotope Methods

Radioisotope and stable isotope tracers are both considered highly accurate techniques used to quantify nutritional parameters (Lo et al. 1981, Janghorbani et al. 1983). Radioisotopes are relatively inexpensive and easily analysed in a gamma or beta counter. They can be, however, very carcinogenic and therefore cannot be used in animals that are to be consumed and present a special risk to humans, especially infants, children and women of child-bearing age. Radioisotope studies require special facilities and all resulting wastes must be collected and stored, often for many years.

Stable isotope tracers are a more recent technique used to determine nutritional parameters. They are usually expensive, but very safe to use. Because of their safety, stable isotopes can be used for long-term studies and do not necessitate elaborate facilities. Samples usually must be activated to their radioactive species by neutron activation analysis, thermal ionization mass spectrometry, or electron impact ionization mass spectrometry (Buckley et al. 1982). Such techniques require very expensive equipment that is not available in many research facilities.

The route of isotope administration selected for a given study depends on the parameters being examined. Absorption can be measured most directly from oral and ruminal doses. However, encapsulation of oral doses can result in a slower rate of absorption than ruminal injections because of a lag time between dosing and appearance of the label in a measureable pool (Hidiroglou and Karpinski 1987). The ruminal route can be the more technically difficult of the two because it requires either precise injection or cannulation. Intravenous and intramuscular injections of the isotope are best suited to quantification of endogenous secretion. From endogenous secretion and concurrent nutrient balance data, estimations of true absorption can be calculated. Hidiroglou and Karpinski (1987) measured absorption and rate of elimination of alpha-tocopherol by four routes of isotope administration. Estimated absorption was greatest for venous, muscular, oral, then ruminal infusion routes, while rates of elimination were highest for venous, oral, then ruminal and muscular routes of administration. Cost and safety are also considerations in the selection of the route of isotope administration. An intravenous dose requires less isotope than other routes, and is therefore less expensive, and in the case of a radioisotope, less hazardous.

Tracer Kinetics and Compartmental Analysis

The use of radiotracers to model the metabolism of an administered substance was first reported by Widmark and Tandberg in 1924. The field of pharmacokinetics was founded in 1937 by Teorell (1937a and b), who compared routes of administration of various drugs and quantified their disappearance in the blood by compartmental analysis. Many years later, in 1960, the use of compartmental analysis as a model for the metabolism of calcium was introduced into the field of mineral metabolism (Aubert and Milhaud). Radiotracers and, less frequently, compartmental analysis, have since been used to model the metabolism of several minerals in animals.

A compartment is defined as a kinetically distinguishable pool (Wagner 1973). By measuring the tracer content of an accessible pool, kinetics is used to deductively ascertain the amount of tracer in nonaccessible regions and its rates of transfer between pools. A compartment need not be an anatomically or physiologically known entity, since volumes of compartments are only apparent volumes. When a metabolite moves from one compartment to another, the rate at which it moves is called the "rate constant". The number of compartments in a system is determined by the number of distinctly different rate constants that can be identified. This in turn depends on the metabolism and physiology of the metabolite.

A system in which transfer of a metabolite occurs at a constant rate is described by zero order kinetics and usually occurs when the transfer system is saturated. A one compartmental model describes first order kinetics and represents a system in which transfer occurs at a rate proportional to concentration. It is described by the following formula:

$$C(t) = A e^{-\beta t}$$

where C is the concentration of the metabolite in plasma, A is the volume of the tracee pool, β is the rate constant and t is time.

A two compartmental model represents a system with two distinct rate constants. It is described by the following equation:

$$C(t) = A e^{-\alpha t} + B e^{-\beta t}$$

The alpha term represents the distribution phase and the beta term the elimination (from sampling medium) phase (Notari 1980).

A three compartmental model is described mathematically as:

$$C(t) = A e^{-\alpha t} + B e^{-\beta t} + G e^{-\gamma t}$$

In this model Notari (1980) suggested that the elimination term from the two compartmental model is divided into beta and gamma terms, where the beta slope represents elimination from the blood and the gamma term represents elimination from deep tissues.

Models with a greater number of compartments are more difficult to interpret, but likely represent a greater splitting of the elimination terms among different tissue groups. It is generally agreed that the preferred compartmental model is the one containing the fewest compartments which adequately describe the data (Notari 1980, Hidioglou and Karpinski 1987). When more than one model can be fitted to the data, values predicted by the models should be compared for their closeness of fit to the observed values. This comparison can be done by several different methods. Plotting observed and predicted values against time for each of the models may reveal major deviations in one of the models. Also, plots of residuals over time for a suitable model should yield a band of randomly scattered points (Landaw and DiStefano 1984). If doubt still remains as to which model to use, a "goodness of fit" test (Draper and Smith, 1981) can quantify statistically the improvement of a higher model over a lower one.

HYPOTHESES

Tracer kinetics and compartmental analysis were chosen for this research project as the most accurate and readily available techniques to test the two primary experimental hypotheses:

1. An increase in dietary protein concentration results in an increase in zinc absorption in calves.
2. Changes in dietary protein concentration alter the rate of endogenous zinc excretion in calves.

The radiotracer, ^{65}Zn , was selected for two reasons. The long half life of ^{65}Zn enables easy measurement of isotope in samples, and ^{65}Zn is the least expensive of available zinc isotopes. An intravenous route of administration allowed a relatively direct measurement of endogenous zinc excretion and lessened the amount of isotope required. The use of stable isotopes was rejected due to cost and the degree of technical difficulty associated with their analysis.

CHAPTER II.

MATERIALS AND METHODS

PROJECT LOCATION AND FACILITIES

The animal component of this research was conducted at the Metabolic Unit of the University of Alberta Experimental Farm, Edmonton, Alberta. During isotope trials the animals were confined indoors in wooden metabolic crates equipped with automatic, overhead feeders. To avoid contamination of feedstuffs and samples with extraneous zinc and copper, only wooden and plastic containers were used for feeding, watering and sample collection. Room temperature was maintained at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Laboratory work was done at the main campus facilities of the Department of Animal Science, University of Alberta.

STUDY ANIMALS

Ten weaned, Holstein bull calves, aged eight to fourteen weeks were acquired. Shortly thereafter, one calf was used in a preliminary trial to test isotope dosage and other experimental procedures. The nine remaining calves were reared in pens and fed a diet of 50% alfalfa hay and 50% commercial dairy ration on a dry matter (DM) basis at a daily rate of 0.03 kg/kg body weight for the three months preceding the experiment. The animals had free choice

access to water, and were handled and allowed exercise daily.

Four of the nine animals (three experimental, one spare) were chosen on the basis of similar ages (approximately 6 months old), weights (200 ± 2 kg), rates of gain (0.78 ± 0.15 kg/d), and temperament for the experiment. A fourteen day pre-experimental period was utilized to adjust calves gradually to metabolic crates and experimental diets. To help reduce physical and psychological stress, calves were exercised daily during all dietary adjustment periods.

DIETS

Calves were randomly allotted to experimental diets containing 8%, 15% and 20% crude protein. Subsequent rotational order was in accordance with a 3 X 3 Latin Square design (Table 1) (Snedecor and Cochran 1967). The diets were initially formulated to contain 12, 18 and 40 $\mu\text{g Zn/g DM}$. However, additional zinc contaminated the concentrates during pelleting. Examination of the effect of zinc level on zinc metabolism at different protein levels was therefore abandoned and the pellets re-mixed to ensure that all diets contained a similar zinc concentration. Diets contained 70% pelleted concentrates and 30% poor quality barley green feed on a DM basis. Dry matter intake was restricted to 2.75% of body weight per

Table 1. Sequence of diets fed to calves during radioisotope trials. LOW - 8% CP, MED - 15% CP, HIGH - 20% CP.

	Duration (Days)	Calf Number		
		1	2	3
Dietary adjustment	10			
Trial 1	5	LOW	MED	HIGH
Dietary adjustment	10			
Trial 2	5	MED	HIGH	LOW
Dietary adjustment	10			
Trial 3	5	HIGH	LOW	MED

day and dispensed in 12 equal portions by automated overhead feeders. Unconsumed feed, which occurred infrequently, was weighed at 12 hour intervals and accounted for in subsequent calculations. Dietary composition and analyses are presented in Table 2.

EXPERIMENTAL PROCEDURES

Approximately 24 hours prior to each experimental period separate catheters for infusion and sampling were placed in the jugular veins of each calf. Catheters placed in the left jugulars were designated for isotope infusion, while catheters placed in the right jugulars were implanted for sampling purposes. All catheters were checked for patency, flushed with heparinized saline and sealed with catheter valves.

^{65}Zn (^{65}Zn) in HCl was obtained from New England Nuclear, Boston, Mass., U.S.A. (S.A. = 10.34 mCi/mL). Aliquots of ^{65}Zn solution were mixed with sterile saline and a measured dose was injected into each calf. The dose for each animal approximated 1.00 $\mu\text{Ci/kg}$ body weight; slight differences from this ratio were recorded for use in later calculations. Blood samples were taken every 10 minutes for the first 100 minutes, followed by samples drawn at 120, 150, 180, 210 and 240 minutes. Subsequent samples were taken at 5, 6, 7, 8, 10, 12, 14, 16, 20, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, 104, 112 and 120

Table 2. Composition (%) and analysis of experimental diets, dry matter basis.

Composition (%)	Crude Protein		
	Low	Medium	High
Barley green feed	30	30	30
Beet pulp	47.7	47.7	47.7
Cornstarch ¹	14.9	8.1	2.3
Rice	5.25	2.9	0.8
Soy flour ²	0.0	4.4	8.3
Egg white powder	0.0	4.4	8.3
Molasses	0.35	0.35	0.35
Minerals and vitamins ^{3,4}	1.75	1.75	1.75
Chemical Analysis			
Crude protein (%)	8.4	15.0	19.6
Gross energy (mcal/kg)	4.2	4.2	4.3
Acid detergent fiber (%)	22.9	23.3	23.5
Zinc (mg/g)	35.4	35.4	33.0
Copper (mg/g)	10.1	10.1	11.1

- 1 Cornstarch to rice ratio maintained at 2.8 : 1.
- 2 Soy flour processed according to Appendix 1.
- 3 Contained per 100g : dicalcium phosphate 1.0g; sodium chloride 0.25g; manganese (in manganese carbonate) 0.004g; Semar 200 (200 mg Se/kg) 0.05; copper (in anhydrous copper sulfate) 0.001g; Vitamins ADE 0.024g.
- 4 Zinc added as zinc chloride.

hours. Blood samples were placed in heparinized test tubes, sealed and stored on ice for a minimum of 20 minutes. Blood was then centrifuged at 2000 x g for 10 minutes and the plasma removed and stored at -4°C.

Fecal grab samples were taken when available for the first 24 hours. The total fecal output of each calf was collected and weighed at 12 hour intervals for five days; each 12 hour portion was mixed and subsampled.

Urine grab samples were taken opportunistically during the sampling periods. Total collection was attempted with a harness and hose system but was unsuccessful. Urine was then collected in plastic trays, and total urine collections were measured for volume every 12 hours. Because of fecal contamination, zinc measurements were performed only on urine grab samples.

SAMPLE HANDLING AND ANALYSIS

The ^{65}Zn activity of plasma samples was measured, using a gamma counter (Model GAM 8000, Beckman Instruments Inc., Fullerton, California). Data were standardized for background radiation in the animals by subtracting the values obtained for pre-infusion samples from all subsequent samples. Total zinc and copper contents of the plasma were analyzed by flame atomic absorption spectrophotometry (Model 4000, Perkin-Elmer Corp., Norwalk, Connecticut) as outlined by Butrimovitz and Purdy (1977).

Fecal samples were placed in pre-weighed paper bags, weighed, and dried in a forced air oven at 45°C until approximately 90% dry. Dried feces were then weighed, extracted from the bags, and ground in a stainless steel grinder. Ground samples were mixed and weighed subsamples analysed for ^{65}Zn emission on the gamma counter and for total zinc and copper content on the spectrophotometer. Spectrophotometric samples were placed in acid-washed (3N HCl) crucibles in a combustion furnace at 500°C for six hours and digested in 6N HCl before measurement.

Because low levels of zinc in urine necessitated condensing the urine, samples were freeze-dried at -60°C in a lyophilizer (Model 50-SRC VirTronics, VirTis Co. Inc., Gardiner, New York). The solids were weighed and gamma emissions determined. Urine samples were prepared for spectrophotometric determination of zinc and copper by the same method used for fecal samples.

PROXIMATE ANALYSIS

Gross energy content of feed and fecal samples was measured by bomb calorimetry (Model 1241 Parr Adiabatic Calorimeter, Parr Instrument Co., Moline, Illinois), crude protein content by the Kjeldahl method (A.O.A.C.) (Model 21237, Labconco Corp., Kansas City, Missouri), and acid detergent fiber (ADF) content according to Goering and Van Soest (1970) (Model 30001, Labconco Corp., Kansas City, Missouri).

CALCULATIONS

Apparent Absorption of Zinc, Copper and Protein

Excretion of zinc, copper and protein for each five day experimental period was calculated by totalling the amount of each nutrient in all 12 hour fecal and urine composite samples. The apparent absorption of each nutrient was calculated according to the following formula (Schryver et al. 1980):

$$\text{Apparent Absorption of X} = \frac{\text{Intake of X} - \text{Fecal X}}{\text{Intake of X}}$$

Endogenous Loss of Zinc

All ^{65}Zn enriched samples were standardized for dose, body weight of calf, assay time (back to a standard time zero so that ^{65}Zn decay in each group of samples was accounted for), ^{65}Zn carryover in periods 2 and 3, and counting container geometry. The specific activity (S.A.) of each plasma sample was calculated according to the following equation (Shipley and Clark 1972):

$$\text{S.A. (cpm/ppm)} = \frac{{}^{65}\text{Zn(cpm)}}{\text{total Zn (ppm)}}$$

The concentration of ^{65}Zn in the plasma at time of injection was calculated from the following equation (modified from Brown et al. 1979):

$$X(0) = Q / q$$

where $X(0)$ is the amount of ^{65}Zn in the plasma per unit of blood (cpm/mL) at the instant after injection, Q is the amount of tracer injected (cpm), and q is the blood volume (mL). The blood volume was calculated by a formula utilizing allometric scaling (Hudson 1985):

$$q = a W^b$$

where w is the weight of the calf and a and b are constants (0.055, 0.99). This formula thus calculates a 200 kg calf to have 10.4L of blood.

Specific activity of plasma versus time for each of the nine data sets was analyzed by non-linear regression using the computer program, BMDP - P3R (Jennrich 1981). Selection of the most suitable number of terms, or compartments, necessary to best describe the observed data was based on several factors. Plots of the natural log of the specific activity of plasma against post-injection time did not describe a straight line for any of the nine data sets. A one compartmental model was therefore rejected. A

four compartmental model could be applied to only five of nine data sets and was therefore rejected. Selection of the most suitable of the remaining two models, the two and three compartmental systems, was based on inspection of plots of residuals versus time and residuals versus predicted values for all data sets. The equation that best described the majority of data sets was then assumed to represent all data sets.

Endogenous loss of zinc was estimated by applying parameters $p_1, p_2, \dots, p_6$, generated by nonlinear regression analysis, in the following equation (modified from Aubert et al. 1963 and Notari 1980):

$$\text{endogenous zinc loss} = \frac{^{65}\text{Zn in feces 24 to 120 h post-injection}}{96} - \int_0^{96} \text{S.A. of } ^{65}\text{Zn (plasma)} dt$$

$$\text{where } \int_0^{96} \text{S.A. (plasma)} dt = \int_0^{96} A e^{-\alpha t} + B e^{-\beta t} + C e^{-\gamma t} dt$$

A 24 hour delay period was presumed to occur from time of secretion of isotope into the intestine until its elimination in feces (Visek et al. 1953). Parameters A, B

and G represent three physiologically distinct zinc pools in the body while parameters alpha, beta and gamma represent transfer rates from pools.

True Absorption of Zinc

Estimations of true zinc absorption for each animal in each treatment were calculated from the formula (Weigand and Kirchgessner 1976):

Percent True Absorption (Zn)

$$= \frac{\text{Zn Intake} - (\text{Fecal Zn} - \text{Endogenous Zn})}{\text{Zn Intake}} \times 100$$

STATISTICAL ANALYSIS

Apparent absorption of zinc, copper and protein, and true absorption of zinc were compared by analysis of variance (ANOVA) for differences between treatments. Analysis of variance for differences between treatments was also performed on endogenous excretion of zinc, expressed in both absolute terms as mg/d and as a percent of total zinc excretion. Analyses by orthogonal polynomials for both linear and quadratic trends were also performed on the apparent absorption, endogenous loss, and true absorption of zinc.

RESULTS

CALF HEALTH

All calves began the experiments in very good health. Throughout the experiments, feed refusals were low, and generally consisted of the roughage component of the diet. During the second experimental period, calf 3 experienced a local infection at the site of the catheter implantation. Although the infection disappeared at the end of that period, it returned during the third period and became systemic ($T = 41.7^{\circ}\text{C}$). The infected calf failed to consume its entire ration and appeared somewhat lethargic. Calves 1 and 2 remained alert and responsive throughout the experiments. All calves experienced some swelling of the joints as the experiments progressed.

PLASMA ZINC

Plasma zinc concentrations were unaffected by protein intake during the course of this study. The mean zinc concentrations for the low, medium and high protein treatments were 0.63 ± 0.05 , 0.63 ± 0.09 , and 0.56 ± 0.16 $\mu\text{g/mL}$ respectively. Occasional difficulty arose in maintaining the patency of jugular catheters for seven days. When absolutely necessary, the infusion catheter was utilized for sampling and, on one occasion, a new catheter was implanted on the second day of the sampling period.

CALCULATIONS

Apparent Absorption of Zinc, Copper and Protein

Within the confines of this study, apparent absorption of zinc did not appear to differ significantly ($P > .05$) between dietary protein treatments (Table 3), as analyzed by both a simple analysis of variance (ANOVA) and by orthogonal polynomials for linear and quadratic trends.

Apparent absorption of copper appeared to increase with dietary protein level but the differences were not statistically significant ($P > .05$) (Table 4).

Apparent absorption of protein was greatly increased ($P \leq .05$) with dietary protein concentration (Table 5).

Experimental period did not affect ($P > .05$) the apparent absorption of zinc, copper or protein.

Model Selection

Visual inspection of plots of the natural log of specific activity versus time indicated that there were changes in the rates of specific activity over time, ruling out a one compartmental model (Figure 1). Four of the nine data sets could not be fitted to a four compartmental model. Hence, the two and three compartmental models were compared for goodness of fit to the observed data. For all nine of the data sets, plots of residuals versus time showed a greater randomness in the three compartmental than in the two compartmental model (Figure 2). In addition,

Table 3. Apparent absorption of zinc (%) by 3 calves at 3 levels of dietary protein.

ANIMAL	DIETARY PROTEIN LEVEL		
	8%	15%	20%
1	7.63	9.43	19.50
2	2.03	33.36	12.33
3	<u>5.24</u>	<u>7.78</u>	<u>38.60</u>
MEANS ¹	4.97	16.86	23.48
St.Dev.	2.30	11.69	11.09

¹ ANOVA showed no difference between treatment means
(F=0.896, P=0.527).

Table 4. Apparent absorption of copper (%) by 3 calves at 3 levels of dietary protein.

ANIMAL	DIETARY PROTEIN LEVEL		
	8%	15%	20%
1	9.1	14.1	25.1
2	7.3	6.3	14.1
3	<u>2.0</u>	<u>36.8</u>	<u>45.9</u>
MEANS ¹	6.1	19.1	28.4
St.Dev.	3.01	12.94	13.19

¹ ANOVA showed no difference between treatment means (F=2.640, P=0.275).

Table 5. Apparent absorption of protein (%) by 3 calves at 3 levels of dietary protein.

ANIMAL	DIETARY PROTEIN LEVEL		
	8%	15%	20%
1	9.1	14.1	25.1
2	7.3	6.3	14.1
3	<u>2.0</u>	<u>36.8</u>	<u>45.9</u>
MEANS ¹	6.1	19.1	28.4
St.Dev.	3.0	12.9	13.2

¹ ANOVA showed difference between means ($F=197.726$, $P=0.005$).

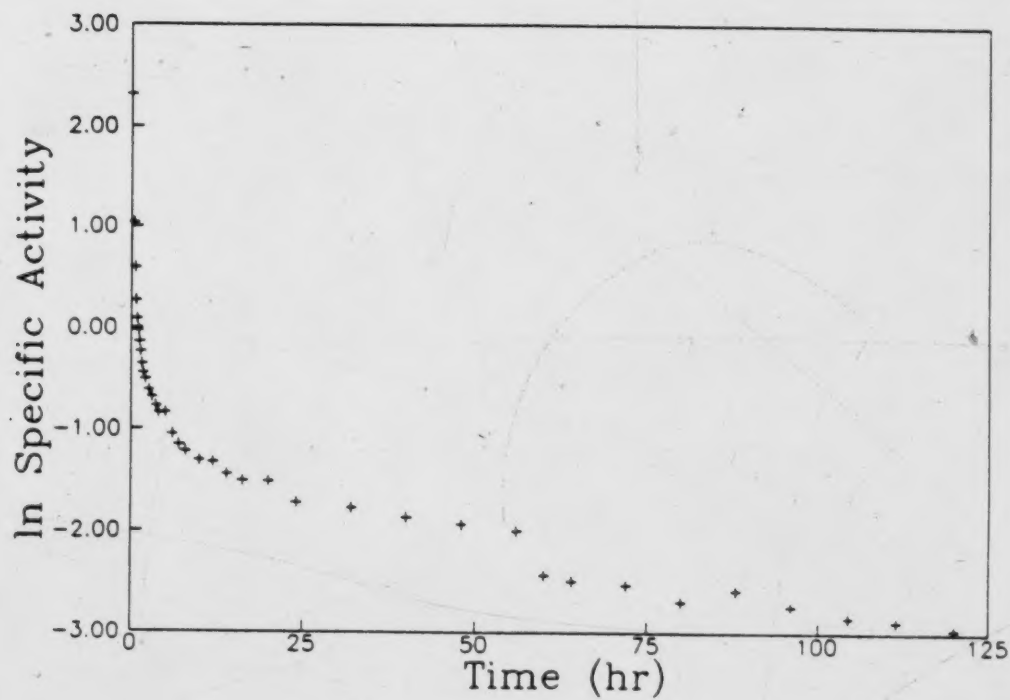


Figure 1. Natural log of specific activity of plasma versus time after injection: representative data set.

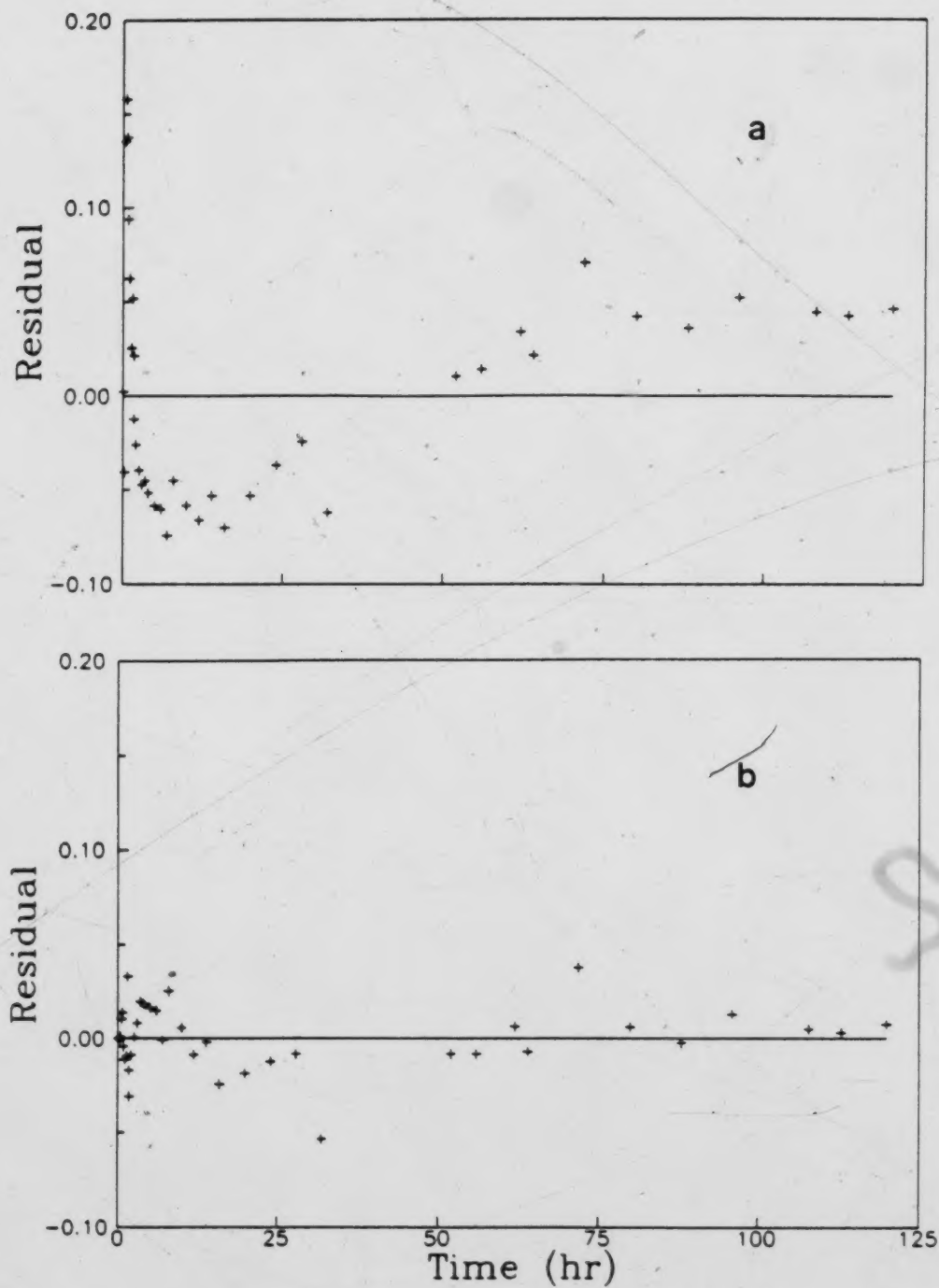


Figure 2. Residuals versus time for 2-compartmental (a) and 3-compartmental (b) analysis of a representative data set.

plots of residuals against predicted values showed a wider distribution of values from the ordinate axis for the two compartmental model (Figure 3) in all data sets. The three compartmental model was therefore chosen to represent the data.

Endogenous Excretion of Zinc

The ^{65}Zn profiles in plasma for each animal in each treatment are presented in Figures 4 to 12. The profiles were best represented by the equation:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} + Ge^{-\gamma t}$$

Parameter estimates for each animal in each treatment are presented in Table 6.

Endogenous excretion of zinc averaged 9.67, 11.59 and 11.71 mg/d for the 8%, 15% and 20% protein treatments respectively (Table 7). Analysis of variance (ANOVA), and analysis by orthogonal polynomials for linear and quadratic trends did not reveal any difference between treatments ($P > .05$). Endogenous excretion of zinc, as expressed as a percent of total zinc excretion, also did not differ ($P > .05$) between treatments (Table 8).

Experimental period did not appear to affect ($P > .05$) the endogenous loss of zinc.

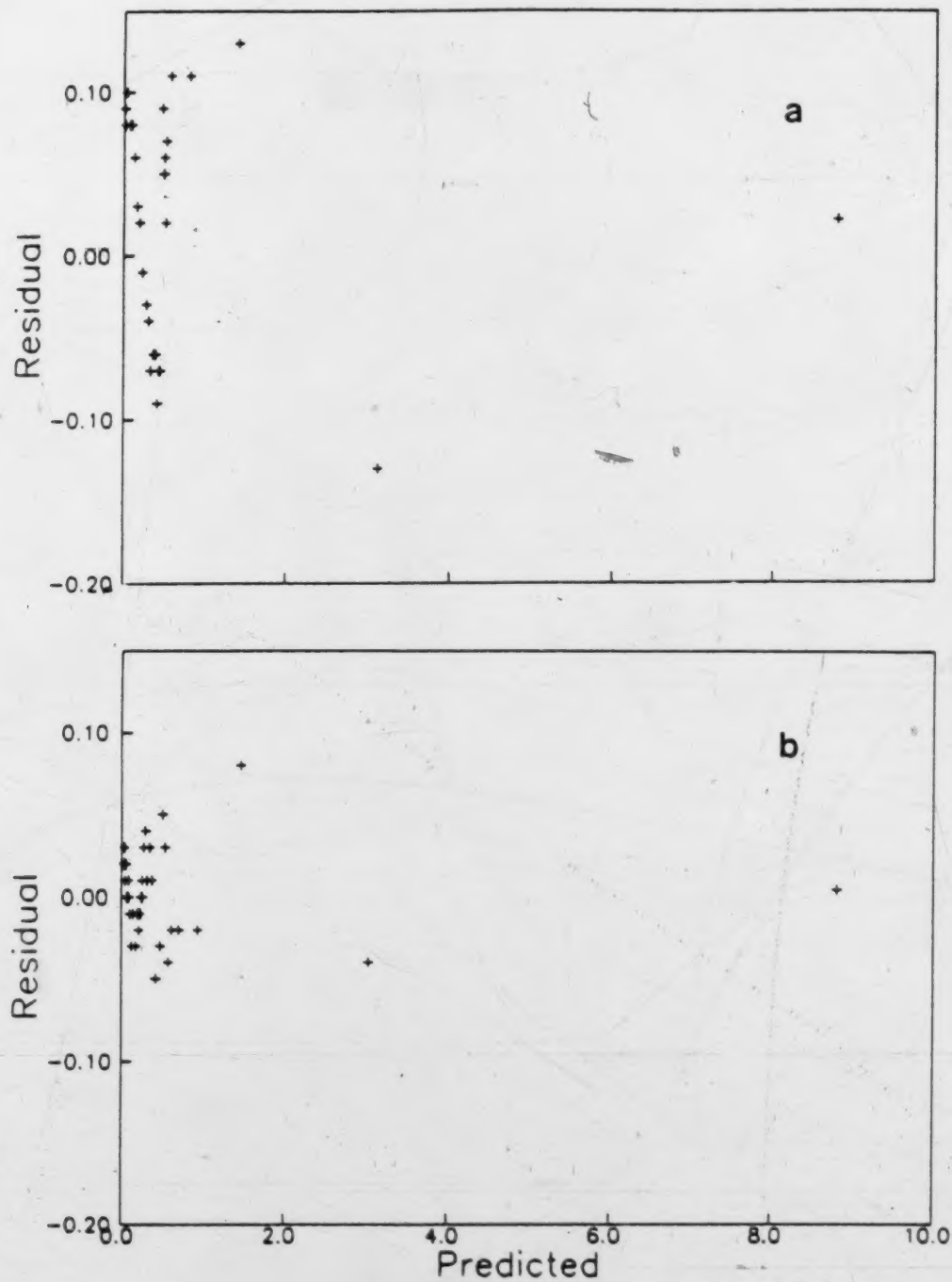


Figure 3. Residuals versus predicted values for 2-compartmental (a) and 3-compartmental (b) analysis of a representative data set.

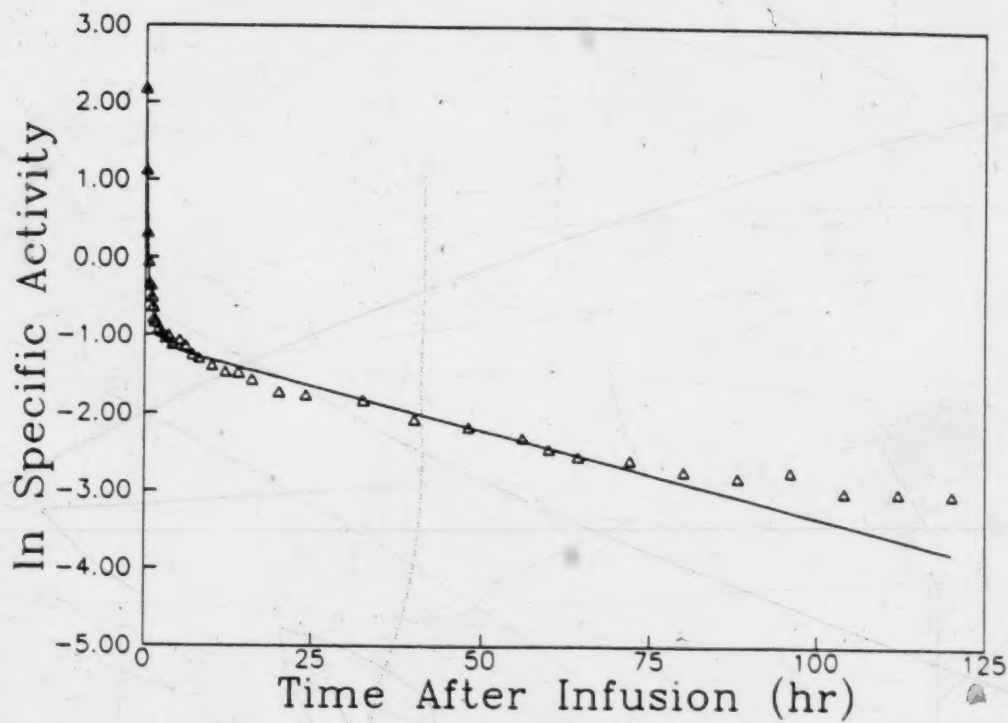


Figure 4. Natural log of specific activity of plasma versus time: low protein diet, animal 1.

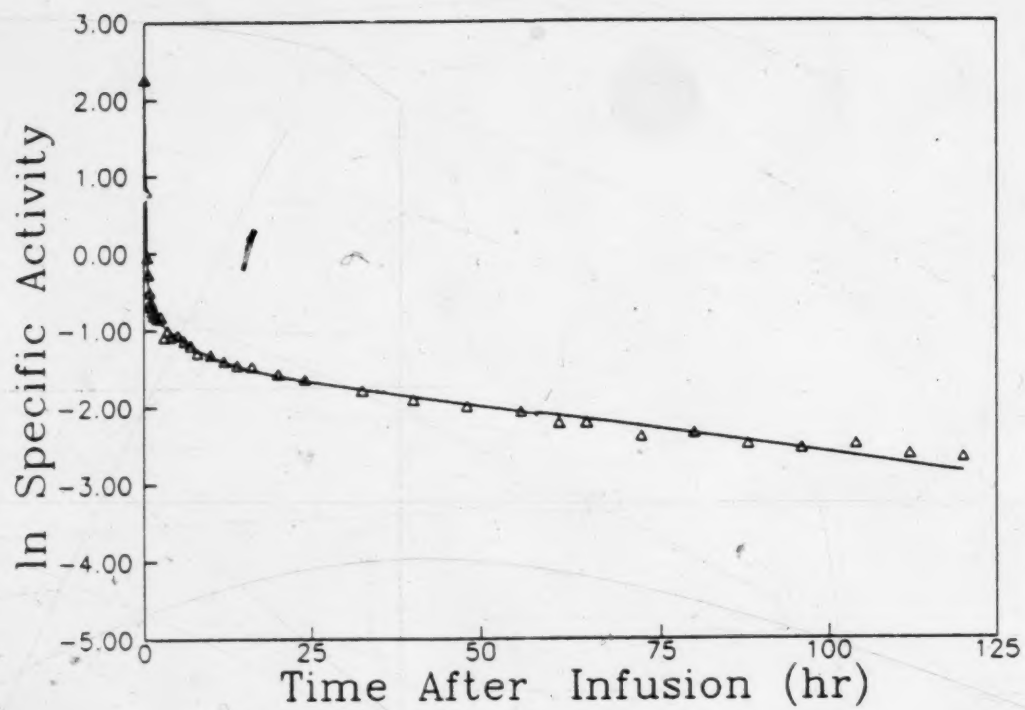


Figure 5. Natural log of specific activity of plasma
versus time: low protein diet, animal 2.

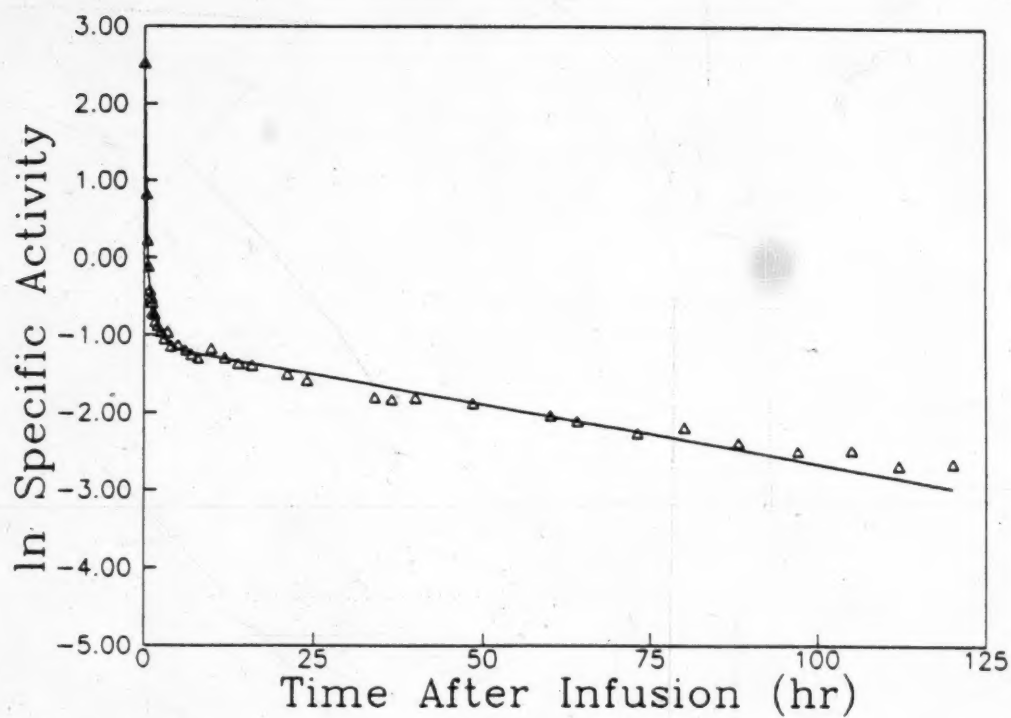


Figure 6. Natural log of specific activity of plasma
versus time: low protein diet, animal 3.

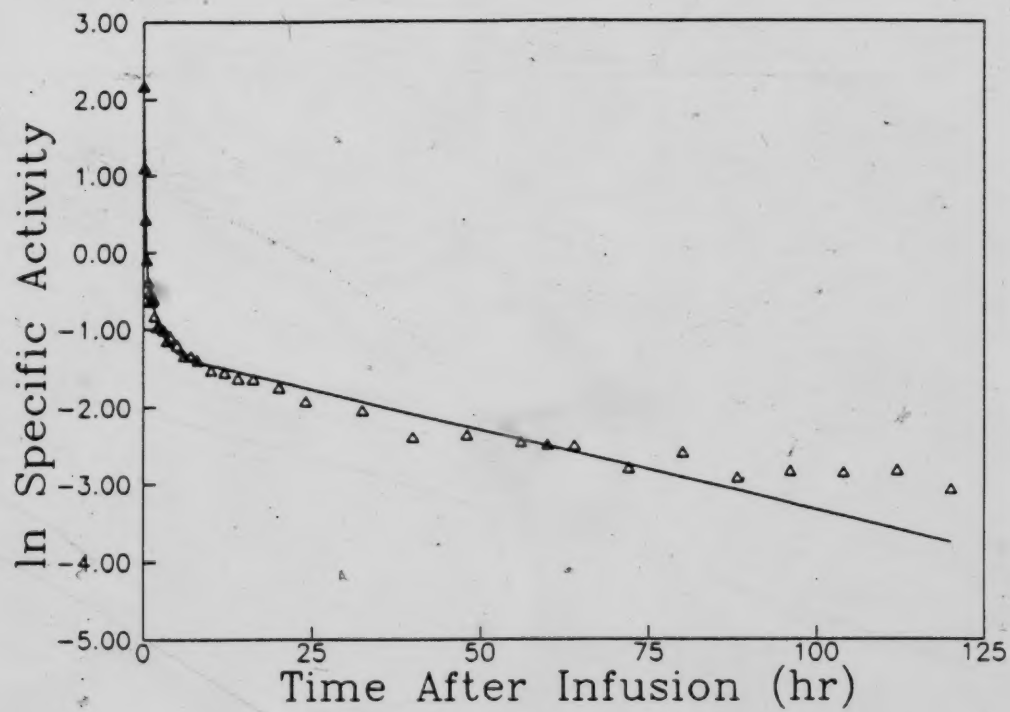


Figure 7. Natural log of specific activity of plasma
versus time: medium protein diet, animal 1.

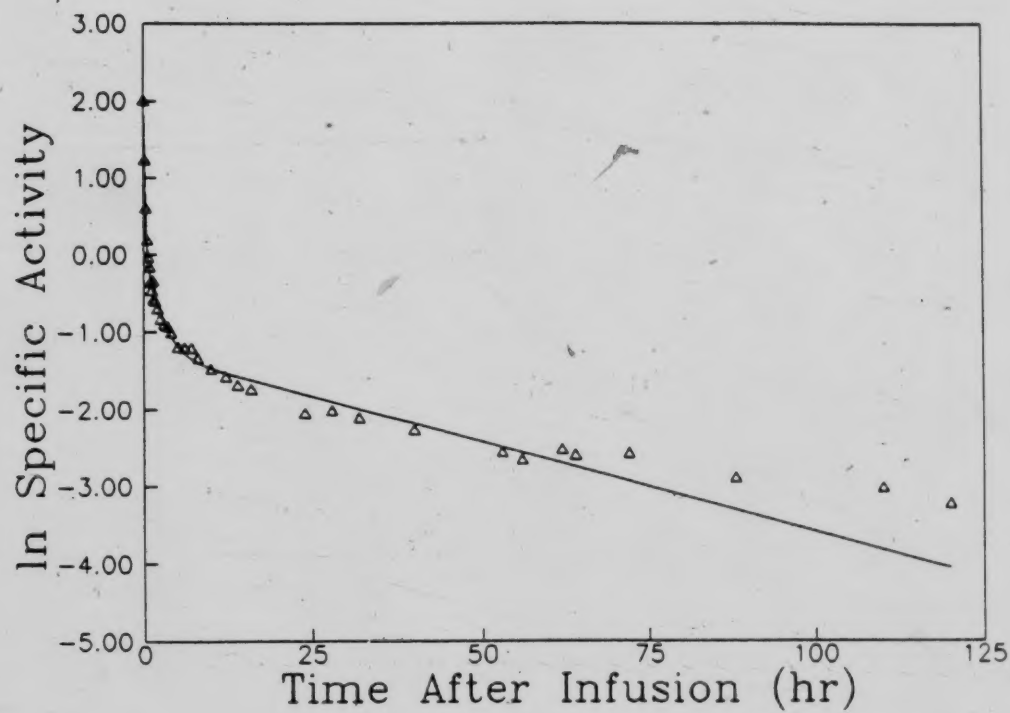


Figure 8. Natural log of specific activity of plasma
versus time: medium protein diet, animal 2.

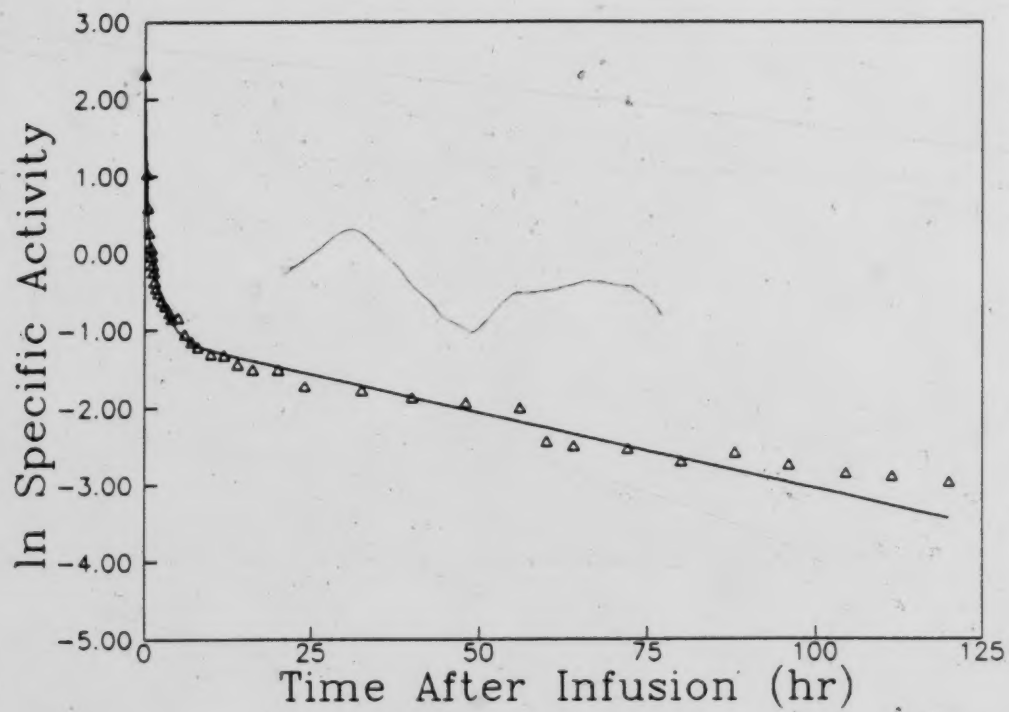


Figure 9. Natural log of specific activity of plasma
versus time: medium protein diet, animal 3.

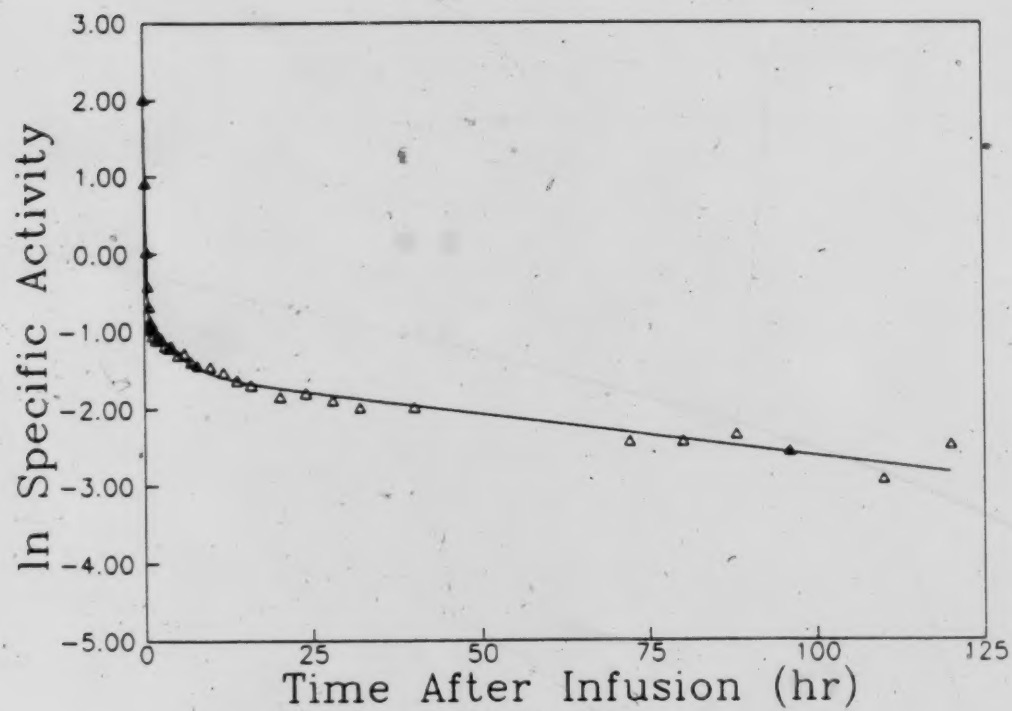


Figure 10. Natural log of specific activity of plasma
versus time: high protein diet, animal 1.

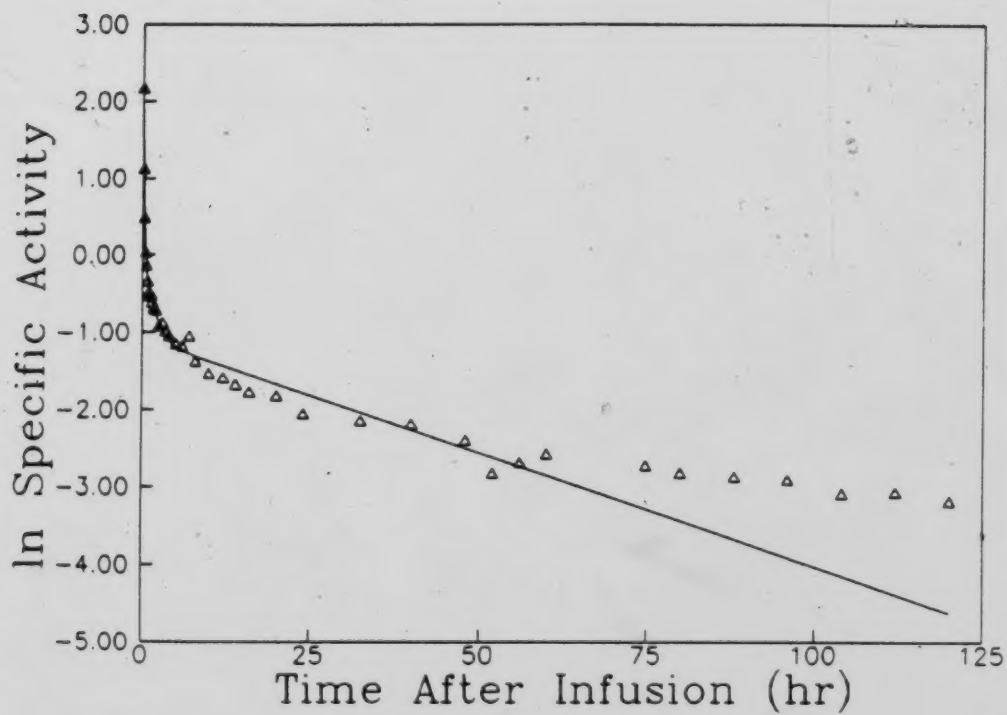


Figure 11. Natural log of specific activity of plasma
versus time: high protein diet, animal 2.

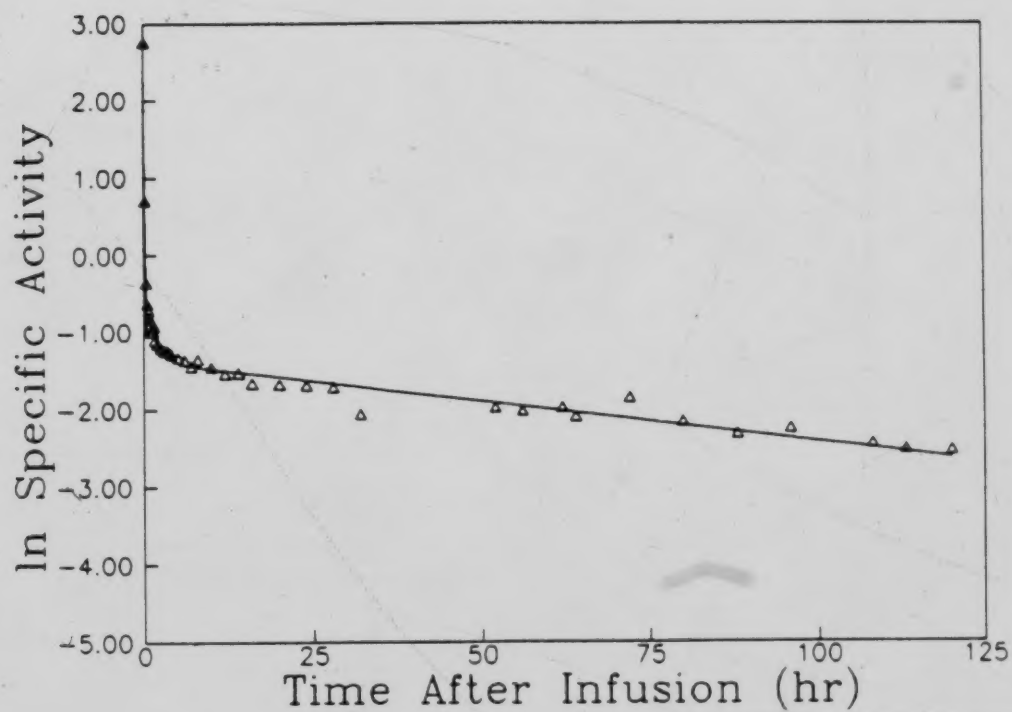


Figure 12. Natural log of specific activity of plasma versus time: high protein diet, animal 3.

Table 6. Experimental equation parameters estimated for profiles of specific activity of ^{65}Zn $C(t)$ in calf plasma after intravenous injection. (Standard Error in parentheses)

Calf No.	Protein Level	$C(t) = A e^{\alpha t} + B e^{\beta t} + G e^{\gamma t}$					
		A	α	B	β	G	γ
1	L	7.7436 (0.092)	-7.7362 (0.152)	0.7396 (0.084)	-1.0751 (0.141)	0.3345 (0.014)	-0.0224 (0.002)
2	L	9.1227 (0.055)	-5.2064 (0.090)	0.0556 (0.045)	-0.1879 (0.082)	0.2529 (0.047)	-0.0122 (0.003)
3	L	11.5433 (0.077)	-6.2346 (0.083)	0.6627 (0.072)	-0.9407 (0.105)	0.3268 (0.009)	-0.0154 (0.001)
1	M	7.8136 (0.068)	-7.6191 (0.129)	0.7041 (0.059)	-0.7691 (0.092)	0.2853 (0.016)	-0.0208 (0.002)
2	M	6.4321 (0.061)	-5.6867 (0.101)	0.7393 (0.049)	-0.5452 (0.069)	0.2875 (0.023)	-0.0234 (0.003)
3	M	8.8461 (0.069)	-5.0141 (0.079)	0.9762 (0.059)	-0.5869 (0.054)	0.3417 (0.017)	-0.0197 (0.002)
1	H	7.0594 (0.026)	-7.2439 (0.066)	0.2303 (0.018)	-0.2529 (0.051)	0.2183 (0.016)	-0.0109 (0.002)
2	H	7.5798 (0.086)	-7.5864 (0.159)	0.8100 (0.075)	-0.8598 (0.114)	0.3406 (0.021)	-0.0295 (0.004)
3	H	15.0864 (0.033)	-13.8527 (0.109)	0.3842 (0.026)	-0.8065 (0.087)	0.2534 (0.007)	-0.0104 (0.001)

Table 7. Absolute endogenous excretion of zinc (mg/d) by 3 calves at 3 levels of dietary protein.

ANIMAL	DIETARY PROTEIN LEVEL		
	8%	15%	20%
1	9.89	11.25	10.39
2	10.11	9.12	10.08
3	<u>9.00</u>	<u>14.40</u>	<u>14.65</u>
MEANS ¹	9.67	11.59	11.71
St.Dev.	0.59	2.66	2.55

¹ ANOVA showed no difference between means (F=2.115, P=0.321).

Table 8. Endogenous excretion of zinc (as % of total zinc excretion) by 3 calves at 3 levels of dietary protein.

ANIMAL	DIETARY PROTEIN LEVEL		
	8%	15%	20%
1	5.79	6.44	6.80
2	4.72	10.47	9.11
3	<u>4.65</u>	<u>5.06</u>	<u>8.85</u>
MEANS ¹	5.05	7.31	8.25
St.Dev.	0.99	2.31	1.03

¹ ANOVA showed no difference between means ($F=1.320$, $P=0.431$).

True Absorption of Zinc

Although low protein intake resulted in a lower true absorption of zinc for all animals (Table 9), true absorption of zinc did not statistically differ ($P > .05$) between treatments as analyzed by both a simple analysis of variance (ANOVA) and by orthogonal polynomials for linear and quadratic trends. Experimental period did not affect ($P > .05$) the true absorption of zinc.

Table 9. True absorption of zinc (%) by 3 calves at 3 levels of dietary protein.

ANIMAL	DIETARY PROTEIN LEVEL		
	8%	15%	20%
1	12.97	15.27	24.97
2	6.66	40.34	20.32
3	<u>9.65</u>	<u>12.42</u>	<u>44.03</u>
MEANS ¹	9.76	22.68	29.77
St.Dev.	2.58	12.54	8.41

¹ ANOVA showed no difference between means (F=1.001, P=0.500).

CHAPTER IV.

DISCUSSION

PLASMA ZINC

Plasma zinc levels of calves in this experiment (mean 0.61 $\mu\text{g/mL}$) were similar to those reported for sheep (Suttle et al. 1982) and ponies (Schryver et al. 1980), but lower than the 0.86 to 1.21 $\mu\text{g/mL}$ concentrations previously reported for Holstein calves (Mills et al. 1967a). The difference from Mill's results is especially surprising because their calves were fed only 25 μg Zn/g DM compared to the 35 μg Zn/g DM fed in this study. It is possible that the lower zinc concentrations in the plasma of my animals are indicative of a marginal zinc deficiency. On the other hand, the value of plasma zinc levels as an indicator of zinc status has been questioned. Several researchers reported that plasma zinc concentration reflected dietary zinc concentrations (Miller et al. 1965a, Mills et al. 1967a, Perry et al. 1968). Others have found no such correlation (Beeson et al. 1977, Schryver et al. 1980). One study compared the effects of zinc deficient and zinc normal diets on calves and reported that zinc concentration in plasma is readily responsive to dietary zinc concentration (Mills et al. 1967a). In another experiment calves fed diets which ranged from normal to nearly toxic in zinc content showed few

significant differences in plasma zinc concentrations (Miller et al. 1970a). It is therefore possible that plasma zinc best reflects changes in zinc status when an animal is fed a zinc deficient diet, but does not reflect variations in dietary zinc that exceed adequate amounts. If the mean plasma zinc level of $0.61 \mu\text{g/mL}$ recorded in this experiment does indeed indicate the presence of a marginal zinc deficiency, the lack of response of plasma zinc concentrations to the different dietary protein treatments may indicate that protein level does not alter the zinc status of calves.

A longer time period than 15 days may be required to detect a change in plasma zinc concentration with protein treatment. Pond and Yen (1984), for example, reported that it took eight weeks for zinc concentration in rat plasma to decrease in response to a protein deficient diet. They also concluded that the decrease in plasma zinc resulted from a depression in plasma albumin, which transports 94% of all plasma zinc (Smith et al. 1979). However, a correlation between plasma albumin and dietary zinc intake has yet to be reported for cattle.

ZINC METABOLISM

No response to an increased concentration of dietary protein was found in the apparent absorption, true

absorption or endogenous excretion of zinc in this study. All calves, however, showed greater apparent and true zinc absorption when fed the medium and high protein diets than the low protein diet (Tables 3 and 9). The very high level of zinc absorption displayed by calf two in the medium protein treatment resulted in a statistically greater difference within treatments than between treatments for the overall experiment. The reason for this apparent anomaly may be related to stress or to zinc deficiency. The medium protein treatment for calf two was the last of three experimental periods for that calf (Table 1). Despite moving the animal into a large, seemingly comfortable metabolic crate, this calf exhibited the most pronounced and progressive joint abnormalities. However, the health of calf two did not appear to affect its protein metabolism (Tables 4). Thus, before the one zinc absorption value that may be an outlier can be discounted, the sensitivity of zinc metabolism to stress should be validated in calves. If indeed the high level of zinc absorption exhibited by calf two while in the medium protein treatment is not due to dietary factors, then a larger sample size might more readily expose this data as an outlier, decrease the effect of individual variation on the overall statistics, and yield a truer test of the effect of dietary protein concentration on zinc absorption.

The animals in this experiment showed a large individual variation in zinc metabolism. Whether similar differences between animals have occurred in other experiments is often difficult to discern. Miller et al. (1966) used only two goats per treatment, and presented neither individual data nor statistics. Suttle et al. (1982) allocated two sheep to each treatment and found either good agreement or poor agreement within treatments depending on the method of calculating the parameters. Miller et al. (1970a, 1970b) used three or fewer calves per treatment and reported considerable variation between individuals. Similarly, Schryver and coworkers (1980) arranged ponies in the same 3 X 3 Latin Square design as was used in this experiment and reported a large standard deviation in zinc absorption means.

Miller (1969) suggested that zinc absorption decreases with age in zinc normal ruminants. Although it is possible that the age of the calves in this experiment may have affected the results, no significant period effect was discernible in any of the parameters of zinc metabolism.

Dietary copper concentrations were maintained at 10 to 11 $\mu\text{g/g}$ DM in all treatments (Table 2). They thus met the level of 10 $\mu\text{g/g}$ DM recommended for dairy cattle by the NRC (1978). It is therefore unlikely that dietary copper was either limiting or selectively interfering with zinc metabolism in any of the treatments in this

experiment. Apparent absorption of copper did not appear to be correlated with increased dietary protein intake (Table 4). This result is similar to that of Van Campen and House (1974) who reported that dietary protein concentration did not affect copper absorption in rats. The effect of dietary protein intake on copper absorption has not been reported previously for cattle.

MODELLING

The three compartmental model was chosen as the most suitable model to fit all nine data points. However, for five of the data sets, a four-compartmental model more accurately described the data (Figure 13). It could not be chosen as the best model because the other four data sets could not be fitted to it.

It is likely that the three-compartments described by the model interact as depicted in Figure 14. The arrangement of this scheme is based on knowledge of blood flow and zinc transport and metabolism. Validation of the accuracy of the model is not possible without sampling less accessible compartments, or pools, and without knowledge of intrinsic volumes and flow rates. The physiological or anatomical identities of the pools are derived solely by deductive logic.

The first compartment is the pool into which the tracer was injected. This is probably the blood plasma but, if

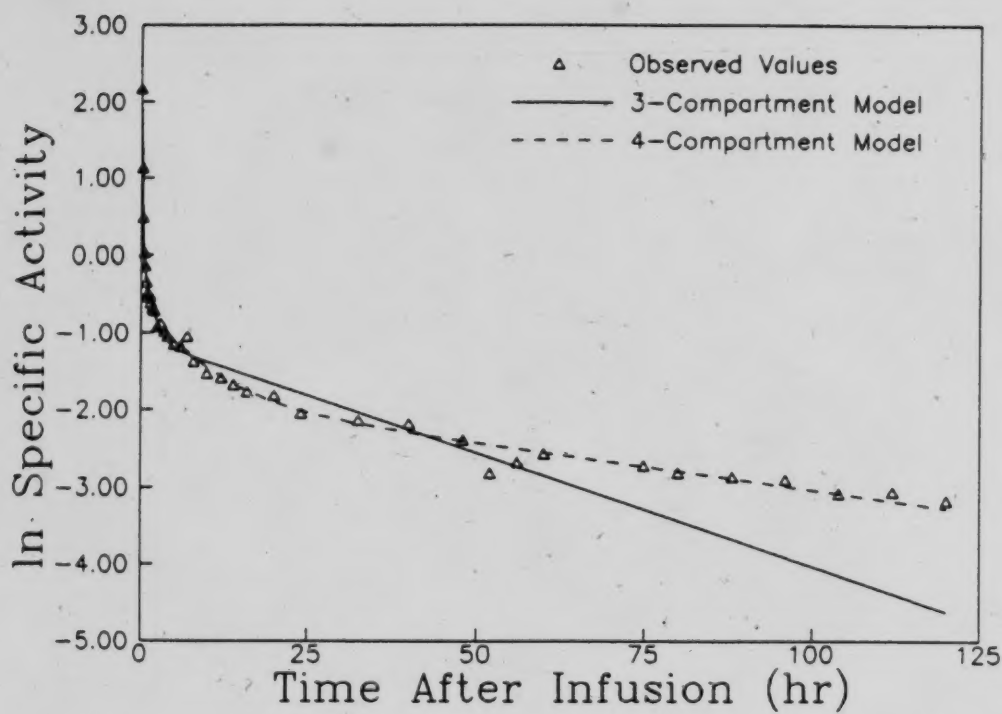


Figure 13. Natural log of specific activity of plasma versus time: observed values and values predicted by three-compartmental and four-compartmental models: animal 2, high protein diet.

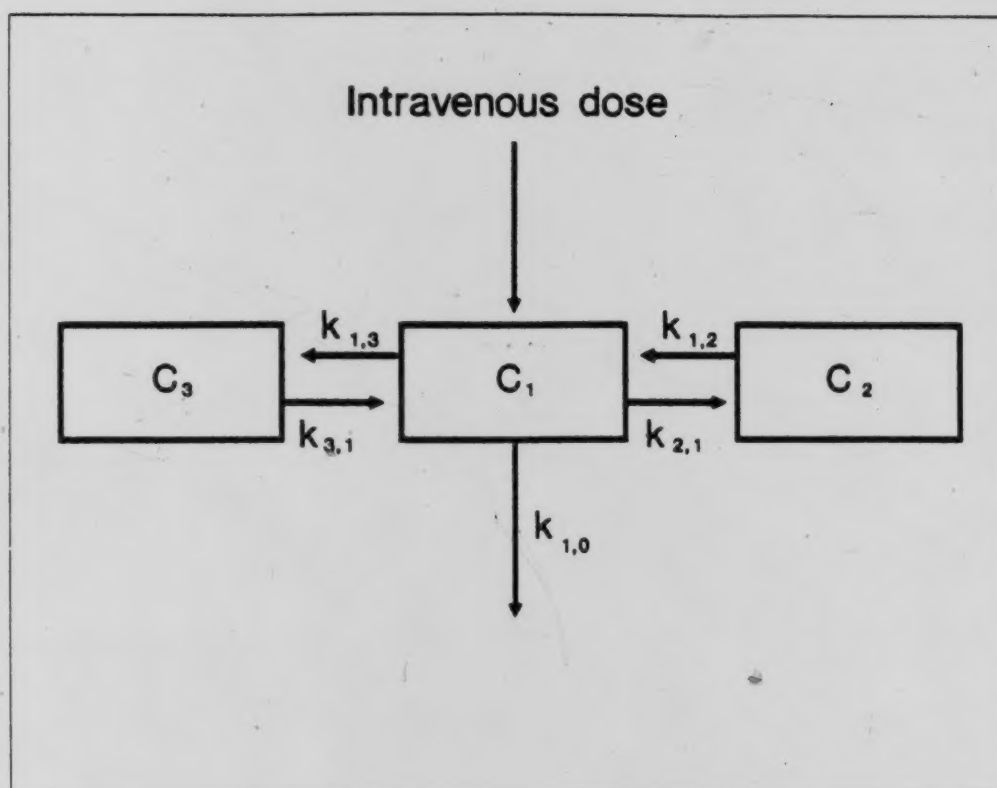


Figure 14. Kinetics of ^{65}Zn as described by a three-compartment exchange system in calves.

the system was not sensitive enough to discern a distinct loss of ^{65}Zn from the plasma to the red blood cells, it may have been the blood. By definition, the most immediate, discernible loss of tracer from the first compartment is to the second compartment. The second compartment would therefore be composed of tissues or organs that most rapidly absorb zinc from the blood or blood plasma. The third compartment absorbs and turns over zinc at a slower rate than do the first two pools.

Red blood cells appear to absorb zinc from plasma in a two-step process in both humans (Foster et al. 1984) and calves (Miller et al. 1970). Foster and coworkers (1984) identified the first step as adsorption to the cell membrane, from whence zinc could either return to the plasma or be absorbed into the cell. The second step was identified as absorption into the cell. Although the turnover rate of red blood cells was characterized by Miller et al. (1970b) as a process measurable in days, the actual rate of zinc absorption was not quantified. It is therefore difficult to determine into which compartment to place the red blood cells. For example, if they absorb zinc very rapidly, they should comprise the second compartment, but if they are slow to absorb zinc, they would be grouped in a more peripheral compartment.

The second compartment very likely contains the liver as this organ has been identified as highly active in zinc

metabolism in calves (Miller et al. 1967, Miller et al. 1970b). A similar conclusion was reached by Foster and coworkers (1984), who calculated that the human liver could extract 67% of plasma zinc in a single passage. Miller et al. (1967) also found that the spleen had a high absorption and turnover rate of zinc; Miller et al. (1970a) identified the pancreas and kidney as similar to liver in zinc absorption and turnover rate. Because a three compartmental model must, by definition, group tissues together into discrete compartments, it is possible that the liver, spleen, pancreas, kidney, and possibly the red blood cells, together comprise the second compartment.

Tissues that are less quickly perfused by plasma zinc, such as bone and hair, and those that strongly control their zinc status, such as heart and striated muscle (Miller et al. 1970a), likely comprise the last compartment of the model. It is possible, however, that their effect on the kinetics of zinc metabolism over a five day sampling period is subtle relative to that of other tissues. If that is the case then, within the confines of a three-compartmental model, the zinc metabolism of this slow, large pool would either be grouped within a pool displaying a faster turnover rate, or would not be characterized within the limits of time in this system.

The exchange of zinc between compartments is probably by transport in plasma. Thus the most plausible depiction

of the three-compartmental system is as illustrated in Figure 14. Although Hidioglou and Karpinski (1987) present numerical solutions for intercompartmental transfer rates to an identical model, references are not provided. Because no literature depicting the calculations necessary to solve these kinetic parameters is as yet available, the model of calf metabolism could not be solved.

SUMMARY

Isotope tracers administered intravenously allowed accurate measurement of zinc excretion from calves. Tracer kinetics and compartmental analysis were used to estimate endogenous zinc losses. Estimates of endogenous loss were added to data derived from a concurrent balance study in order to calculate true zinc absorption. Neither zinc absorption or endogenous zinc excretion increased with dietary protein concentration. It is therefore likely that zinc absorption into the mucosal cells of the intestines of Holstein calves is not affected by dietary protein intake.

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APPENDIX 1

Soy Flour Treatment: Zinc Extraction

Ingredients: 22.7 kg soy flour

Na₂EDTA

NaOH

Apparatus: Large plastic barrel, long stir stick,
plastic siphoning hose, plastic trays,
drying oven.

- Procedure:
1. Place soy flour in barrel.
 2. Fill 2/3rd of barrel with water.
 3. Mix thoroughly. Buffer to pH 4.3.
 4. Add 0.412 kg Na₂EDTA.
 5. Mix. Allow to settle for 12 h.
 6. Siphon off supernate.
 7. Repeat steps 2 to 6, five times.
 8. Place precipitate on plastic trays.
 9. Place trays in drying oven at 45°C.
 10. Dry to 90% DM. Regrind.

Procedure greatly modified from: Davis, P.N., L.C. Norris
and F.H. Kratzer. 1962. Iron deficiency studies in chicks
using treated isolated soybean protein diets. J.Nutr.
78:445-453.